



Research article

Genetic conservation of bamboo in Loei province, Thailand: Identification, distribution and genetic diversity

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Abstract

Over the past decade, deforestation has triggered an environmental crisis causing the extinction of some native plants worldwide. Therefore, in Thailand, a plant genetic conservation effort, as a Royal Project under the initiative of Princess Maha Chakri Sirindhorn, has been established with the ultimate goal of preserving biodiversity, conservation and preventing extinction of wild plants in Thailand. As part of this Royal Project, the current research surveyed bamboo distribution, identified bamboo species and evaluated the genetic diversity of native bamboo in three types of terrain (river basins, relatively flat areas and mountainous regions) in Loei province where bamboo diversity is rich. Bamboo was identified at the species level based on morphological characteristics (shape, color and culm length, rhizomes, shoots, leaves, flying shoots, culms, limbs, nodes, internodes, buds, hairs and sheaths). There were 34 bamboo species in Loei in 8 genera comprising 11 species of *Bambusa*, 6 of *Dendrocalamus*, 3 of *Schizostachyum*, 2 of *Gigantochloa*, *Thyrsostachys*, *Vietnamosasa* and *Neohouzeaua*, 1 of *Melocalamus* and 5 unknown genera. Different morphological characteristics of 22 bamboo species in 7 genera were selected from the 34 bamboo species for evaluation of their genetic diversity using 10 amplified fragment length polymorphism markers. The resulting information was used to construct a phylogenetic tree in which the 22 bamboo species could be divided into three groups. The phylogenetic tree had Jaccard similarity coefficients in the range 0.1273–0.7281 with a cophenetic correlation of 0.9279, indicating a high degree of grouping.

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Introduction

Bamboo is a perennial plant in the Poaceae family and subfamily Bambusoideae that grows as tall, tree-like grass with long life cycles (Wong, 2004). Bamboo, as both woody and fast-growing grass types, is naturally distributed all over the world, occurring in the tropical, subtropical and temperate regions of Asia, South America and Africa (Londoño, 1998; Judziewicz et al., 1999; Bystriakova et al., 2004). There are 80–90 genera of bamboo with 1,500 species worldwide (Sungkaew et al., 2014). That have had multiple uses for many centuries. For example, bamboo shoots are used for food and herbs in China, India and Thailand (Sungkaew et al., 2014), while the stems have been fashioned into tools for daily life, musical instruments, food containers, toys, fishing devices, farm implements, fences, bicycles (Bernice, 2015), crutches, building materials (Munir, 2015) and furniture (Lobovikov et al., 2007). Bamboo contributes to environmental equilibrium by serving as a carbon sink, providing oxygen, preventing soil erosion, being a source of organic matter, inhibiting soil degradation and improving the overall stability of sloping land (Kaushal et al., 2021). There are 13 genera with 69 species of bamboo in the evergreen and mixed forests of Thailand of which 62 have useful purposes, with 12 genera containing 45 species being native, while others are imported (Sungkaew et al., 2014).

Basic knowledge on the biology of bamboo is lacking due to its unusual life cycle with the vegetative phase ranging from 1 to 120 years (Ramanayake et al., 2007). Furthermore, morphological data such as inflorescence type that has been used in defining genera, is not easy to interpret (Ramanayake et al., 2007). Therefore, an alternative could be the use of molecular systematics or genetic investigation based on methods of identifying DNA polymorphisms between individuals or species to overcome the problem of the taxonomic identification of bamboos.

Loei is one of the richest areas of bamboo diversity in Northeastern Thailand due to its varied topography consisting of relatively flat areas, river basins and mountainous forests (Yospanya, 2007). For more than a decade, these areas have been seriously deforested through slash-and-burn agriculture. Therefore, the deforestation crisis in Loei province has begun to be addressed with a new round of environmental awareness. Native plants, such as bamboo, face extinction and are critically endangered. This is certainly the case in Loei where bamboo makes up 50% of the total threatened species (Thailand Forest Survey Team, 2009). The conserved forest area has been reduced by at least 48.3% over the past 15 years

due to deforestation (Office of Agricultural Economics, 2021). Worldwide, 450 species of bamboo are at risk of extinction (Lobovikov et al., 2007). The objectives of the plant genetic conservation project under the initiative of Her Royal Highness Princess Maha Chakri Sirindhorn are to develop professionals with knowledge of approaches to plant genetic conservation. This will address the crisis of vanishing wild plants and preserve the genome of endangered species. Therefore, augmentation of the bamboo genome database will be applied in conservation and rehabilitation efforts in Thailand. The research starts with surveying, gathering, and analyzing bamboo varieties and their diversity. Consequently, biodiversity, grouping/clustering, morphological and genetic characteristics of native bamboo need to be elucidated, with genetic diversity assessment among selected species being measured using DNA markers and amplified fragment length polymorphism (AFLP).

Materials and Methods

Survey of bamboo distribution and identification of species using morphological characteristics

Initially in exploratory research, areas of native bamboo areas in Loei were randomly surveyed, as shown on the map in Fig. 1. Three bamboo samples from each of 34 species were collected from three types of terrain comprising: a) relatively flat areas in Muang, Chiangkan and Thali districts; b) the river basins of Muang and Chiangkan districts; and c) the mountainous regions of Muang, Phurua, Thali and Dansai districts. A completely randomized design with three replicates was applied. The morphological characteristics of Loei bamboo (culm color, culm length, rhizomes, shoots, leaves, flying shoots, culms, limbs/branches, nodes, internodes, buds, hairs and sheaths) were examined following Seethalakshmi and Muktesh (1998), Ohrnberger (1999), Wong (2004), Sungkaew et al. (2014), American Bamboo Society (2016) and Vorontsova et al. (2016).

Genetic diversity

Sample collection

Only 22 local bamboo species with different morphological characteristics were identified for genetic diversity in this study. Randomized samples were collected of young leaves from the 22 species. Then the samples were brought to the DNA Technology Laboratory of Kasetsart University, Bangkok, Thailand, for assessment of their genetic diversity.



Fig. 1 Map of Loei province, Thailand showing surveyed areas in bamboo distribution in districts of Chaingkan (a), Thali (b), Phurua (c), Muang (d) and Dan Sai (e) (Source: Maps of the World, 2018)

DNA extraction

DNA was extracted from these leaves using the method of Lodhi et al. (1994) with slight modification of the reagent volumes. The extracts were incubated with 20 μL of 1 $\text{mg}\cdot\text{mL}^{-1}$ RNase A at 37°C for 30 min; then, they were kept at 4°C for subsequent experimentation. The concentration of DNA was determined by comparing lambda DNA using agarose gel electrophoresis. The stained gel was visualized under ultraviolet light. The DNA concentration was estimated by comparing the samples with the intensity of DNA markers. The extracted DNA samples were diluted to 1 $\text{ng}\cdot\mu\text{L}^{-1}$ and kept for further use.

Amplified fragment length polymorphism

The DNA was first digested with a restriction endonuclease comprising *EcoRI* and *MseI*. Next, an adapter was ligated to the ends of the digested DNA. In total, 10 randomized AFLP primer combinations were used with the extracted DNA to analyze genetic diversity. The primer names used for preamplification were ER-A, MS-C and MS-G with the sequences of AGACTGCGTACCAATTCA, GATGAGTCCTGAGTAAC and GATGAGTCCTGAGTAAG, respectively. However, the AFLP primer combinations for selective amplification were composed of forward and reverse primers with three selective bases at the 3' end. Primer 1 was ER-AAC with the sequence AGACTGCGTACCAATTCAAC, 20 bp. It was paired with primer 2 of the 10 primers and each was 19 bp. The 10 primers designed by the DNA Technology

Laboratory, Kasetsart University, Bangkok, Thailand were MS-CCA, MS-CCC, MS-CCG, MS-CCT, MS-CAA, MS-CAC, MS-CAG, MS-GAC, MS-GAG and MS-GTG with the sequences of GATGAGTCCTGAGTAACCA, GATGAGTCCTGAGTAACCC, GATGAGTCCTGAGTAACCG, GATGAGTCCTGAGTAACCT, GATGAGTCCTGAGTAACAA, GATGAGTCCTGAGTAACAC, GATGAGTCCTGAGTAACAG, GATGAGTCCTGAGTAAGAC, GATGAGTCCTGAGTAAGAG and GATGAGTCCTGAGTAAGTG, respectively. Polymerase chain reaction (PCR) was used following Kitsanachandee et al. (2013). PCR was performed and the DNA was amplified in a GeneAmp PCR System 9700. The PCR products were separated on a 5% denaturing polyacrylamide gel and visualized using silver staining.

Phylogenetic analysis

The DNA bands from AFLP were visualized and scored using 1 for a band and 0 for no band at the same locus or DNA size. The data were prepared from 179 monomorphic bands with means of 17.9 bands per marker and 8.14 bands per bamboo species. A phylogenetic tree showing genetic diversity among the bamboo species was constructed based on the Euclidean distance dissimilarity method and grouping using the unweighted pair group method with arithmetic mean using the NTSYS 2.02J software. Jaccard similarity and cophenetic correlation were also calculated from the polymorphism using NTSYS 2.02J.

Results and Discussion

Survey of bamboo distribution and identification of bamboo species using morphological characteristics

Flat terrain areas

This study was the first published attempt to estimate the bamboo resources in Loei province. In total, there were 19 bamboo species from 6 genera and 1 unidentified genus, as indicated in Table 1. General views of the bamboo genera are shown in Fig. 2. *Bambusa* was represented by seven species: *B. bambos*, *B. blumeana*, *B. nutans*, *B. vulgaris* f. *waminii*, *B. burmanica*, *B. vulgaris* and *B. multiplex*. *B. bambos* (giant thorny bamboo) was widely distributed in every district of Loei and is a very densely tufted and large woody bamboo with root thorns from the nodes which erect culms that are slightly bending outward above with dull medium green, durable, cylindrical culm internodes, hollow, thick walls having a lumen, and one dominant branch (Seethalakshmi and Muktesh, 1998).

B. blumeana, a woody bamboo similar to *B. bambos*, has tall culms with root thorns from the nodes, terete-glabrous young internodes, branching present from the base upward, and hispid culm sheaths with dark brown hairs on the back of the culm-sheaths (Gilliland, 1971 and Sungkaew et al., 2014). *B. nutans*, a perennial, is a medium woody bamboo, loosely culmed with much branching above, with aerial roots from the nodes and glabrous culm nodes. It has straight culms, terete internodes with a small lumen that is smooth and green with a matte surface that has white-ringed hairs below the nodes and a culm sheath with appressed hairs that are black when pubescent. *B. vulgaris* (Sangkham), has characteristics as described in the studies of Seethalakshmi and Muktesh (1998) and Sungkaew et al. (2014). *B. burmanica*, a large woody bamboo similar to other *Bambusa* species, is dull green without nodal roots. It is durable with a nearly solid culm and small lumen that is marked by a white ring of hairs below the nodes. The green color becomes yellow at the edges of the culm-sheaths of young bamboo having a culm sheath that is hairy at the margins and covered with appressed golden hairs on the sides that are glabrous on the back.

Table 1 Surveyed bamboo species in flat terrain areas of Loei province

No.	Local name	Species	Distribution
<i>Bambusa</i> genus			
1	Pa	<i>B. bambos</i>	Hills
2	Seesook	<i>B. blumeana</i>	Hills, road side, and waterside
3	Bongban	<i>B. nutans</i>	Hills
4	Namtao	<i>B. vulgaris</i> f. <i>waminii</i>	General areas
5	Bongwanmuangloei	<i>B. burmanica</i>	Hills, upland, and farm
6	Luang, Sangkum	<i>B. vulgaris</i>	Hills and riverside
7	Sangprailek	<i>B. multiplex</i>	General areas
<i>Dendrocalamus</i> genus			
8	Tong	<i>D. asper</i>	Little found in hilly terrain
9	Bongyai	<i>D. brandisii</i>	Hills and waterside
10	Sang	<i>D. membranaceus</i>	Hills in Muang district
11	Hog	<i>D. hamiltonii</i>	Little found in hilly terrain in Muang district
<i>Gigantochloa</i> genus			
12	Rai	<i>G. albociliata</i>	General areas
13	Damkwan	<i>G. ligulata</i>	Little found in hilly terrain and conservation areas
<i>Thyrsostachys</i> genus			
14	Roug	<i>T. siamensis</i>	Hilly terrain
15	Rougdam	<i>T. oliveri</i>	Hilly terrain
<i>Vietnamosasa</i> genus			
16	Jode	<i>V. ciliata</i>	General areas
<i>Schizostachyum</i> genus			
17	Kaolam	<i>S. pergracile</i>	Hilly terrain
18	Seetong	<i>S. brachycladum</i>	Houses and gardens
Unidentified genus			
19	Sangong	-	Hilly terrain and riversides

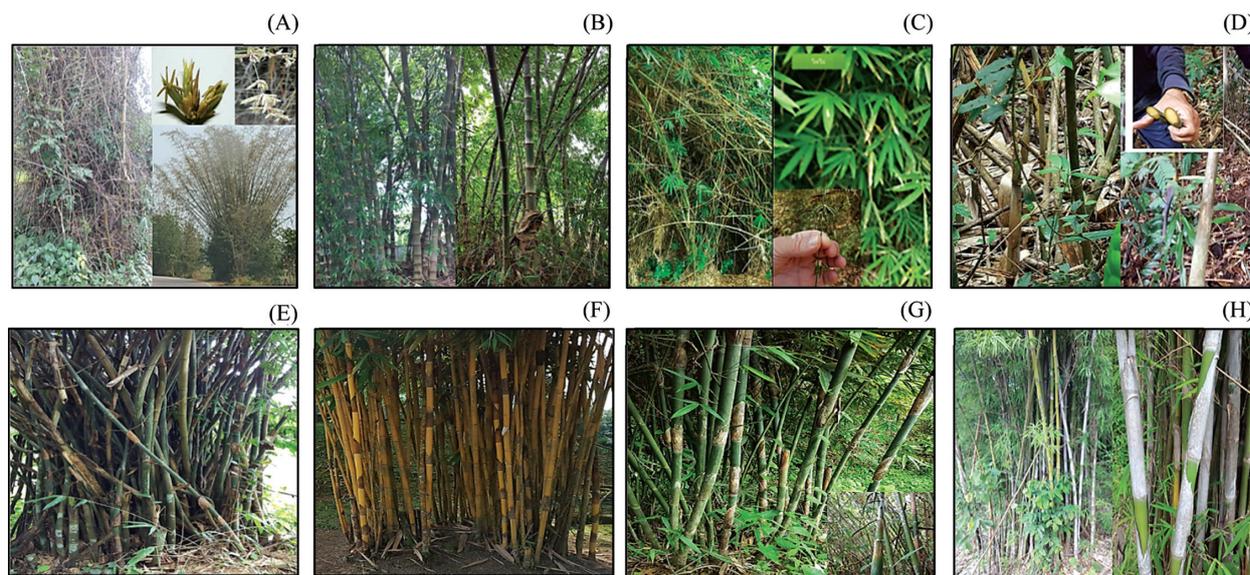


Fig. 2 General views of bamboo genera from distribution survey in Loei province, Thailand: (A) *Bambusa*; (B) *Dendrocalamus*; (C) *Gigantochloa*; (D) *Melocalamus*; (E) *Neohuazeaua*; (F) *Phyllostachys*; (G) *Schizostachys*; (H) *Thyrsostachys*

The four identified species of the *Dendrocalamus* genus were: *D. asper*, *D. brandisii*, *D. hamiltonii* and *D. membranaceus*. An obvious phenotypic trait of *D. asper* was its very large and strong culms, with aerial roots from the node and durable thick walls with deciduous culm sheaths with initially sparse hairy leaf sheaths and the presence of buds or branches on the lower culm similarly to other reports (Dransfield and Widjaja, 1995; Sungkaew et al., 2014). *D. brandisii* is a large evergreen tufted bamboo with ashy-gray to greenish-gray erect culms, slightly swollen nodes that are thick and leathery, with white pubescence on the backs of the culm sheaths that are twice as long as wide, coriaceous, pubescent with white hairs are concave at the apex. *D. hamiltonii*, with its short-necked rhizome, has erect and arching culms outward on top with root dots on the nodes and without limbs on the lower culms. When pubescent, it has terete culm internodes with a small lumen. It is dark green and thick-walled, with rough culm sheaths that become glabrous or with dark brown rigid hairs, small auricles and triangular culm sheaths. It is ligule along the entire culm sheath with large foliage leaf blades when mature. *D. membranaceus*, a pachymorph rhizome bamboo, is moderate-sized with straight culms, culm nodes slightly prominent on the culm covering with a farinose ring just below the sheath scar when young. It has a dark green culm at maturity, with nodal rings and hispid lead sheaths. The basal nodes have slender and leafy rootlets, with drooping upper limbs. They are glabrous outside or with appressed dark brown hairs and narrowed upward culm-sheaths (Seethalakshmi and Muktesh, 1998).

On flat terrain, there were two species each of the genera *Gigantochloa*, *Thyrsostachys* and *Schizostachys* comprising *G. albociliata*, *G. ligulata*, *T. siamensis*, *T. oliveri*, *S. pergracile* and *S. brachycladum*. Interestingly, *G. albociliata* is caespitose with pachymorphs. It has erect and densely tufted culms with pendulous tips that are distally hispid, terete culm internodes with a small lumen with moderately thick walls that are grayish-green with white stripes and prominent culm nodes raised in at the base of the sheath scar (Seethalakshmi and Muktesh, 1998). Its appressed tawny hairs are absent when the bamboo is young. Similarly, *G. ligulata*, with short rhizomes, has young internodes with scattered dark or short white hairs (Gilliland, 1971) with the limbs developing from upper nodes singly or with several limbs per node. They are persistent, have culms with a small lumen that are concolorous medium-to-light green, distally pilose, crustaceous, with yellow-green culm leaves (Rattamane, 2014) and buds or branches are absent from the lower culms. Within the genus *Thyrsostachys*, the culms of *T. siamensis*, and *T. oliveri* present pachymorph rhizomes that are thick with small lumina. In Loei, *T. siamensis* is more prevalent than *T. oliveri* on flat terrain. Additionally, the internodes of young culms are dense and dark olive green in *T. oliveri*, while *T. siamensis* is glabrous or initially hairy covered with white fuzz on the upper part, or a glossy medium green culm, persistent culm sheath, hairy throughout with lateral branches in a horizontal line with one dominant branch. The phenotypic characteristics of *Schizostachys*, composed of *S. brachycladum* and *S. pergracile*, are its pachymorph

rhizomes, stiffly erect culms that are golden or dark in color, terete culm internodes, with yellowish brown culm leaf sheathes, red-brown hairs, ligule hairy culm leaves and blades that are broadly triangular to dome-shaped leaves (Dransfield and Widjaja, 1995). In the current study, *Vietnamosasa ciliata* had leptomorph elongated-rhizomes, dendroid lateral limbs, many complementary branches, with pubescent, leaf sheath hair ciliate and striated veined leaf-sheaths.

The lowlands and relatively flat terrain are suitable habitats for these native bamboo species with different growth rates, development patterns and a larger size than those growing in mountainous areas. The identified species occurred naturally or were cultivated in various locations in the relatively flat areas such as in the foothills, along roadsides, on farms, at houses and in gardens, as well as along the riverside and at water edges. Their abundance, diversity and evenness were correlated with topography, habitat, variety, advantage, method of propagation, uses and human preferences (data not shown). Most of the local bamboo in this areas were native varieties, such as *B. blumeana*, *B. vulgaris* and *B. bambos*, with *B. vulgaris* and *S. brachycladum* being grown as garden decorations. *B. multiplex* was grown for culms that are used in building materials or for construction and in agricultural applications due to its higher durability and flexibility than other species. In Loei, the young shoots of *G. albociliata* are used in traditional foods for their bitter taste and most people grow bamboo for fresh shoots and use them for food.

River basin areas

Four bamboo genera comprising 11 species were identified and are listed in Table 2. There were five species from the *Bambusa* genus (*B. bambos*, *B. blumeana*, *B. nutans*, *B. vulgaris* and *B. multiplex*), of which there were three sub-species, commonly known as Sangprailek, Lieng and Sangpraiyai, having similar morphologies and phylogenetics. One of each species in the *Dendrocalamus*, *Gigantochloa* and *Thyrsostachys* genera and 1 unidentified genus were *D. hamiltonii*, *G. ligulata* and *T. siamensis*, respectively. Only the Sangong variety could not be identified although it appeared to have similar characteristics to *B. multiplex*, with erect culms and dark green, glittering bamboo, except for the culms widening on the upper part of the plants, with few dense limbs at the lower nodes. Most native bamboos were wild plants along the riverside. The phenotypic traits of all genera in the river basin areas were closely related to those found in the relatively flat areas, except in size. *B. multiplex* (Lieng), had similar characteristics to those reported by Sungkaew et al. (2014) and Goh et al. (2018) remaining under the provisional names, *B. multiplex* and *Arundo multiplex*. Among species of the genus, *Bambusa*, *B. blumeana*, *B. nutans* and *B. vulgaris* occurred more naturally than Sangpraiyai (*B. multiplex* and *B. bambos*). After more than a decade, the original habitat of Sangpraiyai in mountainous areas has declined because of planting of crops such as para rubber. Many bamboo species in Loei appeared to have become extinct based on not being identified in the current

Table 2 Surveyed bamboo species in river basins of Loei province

No.	Local name	Species	Distribution
<i>Bambusa</i> genus			
1	Pa	<i>B. bambos</i>	Waterside and tributary edges
2	Seesook	<i>B. blumeana</i>	Waterside, pond side, road side and creek side
3	Bongban	<i>B. nutans</i>	Waterside and creek side
4	Luang, Sangkum	<i>B. vulgaris</i>	Loei riverside, tributary
5	Sangprailek	<i>B. multiplex</i>	Along tributaries, riverside, waterside and farm
6	Lieng	<i>B. multiplex</i>	Along tributaries, riverside and farm
7	Sangpraiyai	<i>B. multiplex</i>	Along tributaries, riverside, waterside and farm in Muang district
<i>Dendrocalamus</i> genus			
8	Hog	<i>D. hamiltonii</i>	Little found on tributary riverside
<i>Gigantochloa</i> genus			
9	Damkwan	<i>G. ligulata</i>	Along tributaries, riverside
<i>Thyrsostachys</i> genus			
10	Roug	<i>T. siamensis</i>	Along tributaries, riverside in the hilly areas, waterside and ridges
Unidentified genus			
11	Sangong	-	Along tributaries, riverside, waterside and streams

study. *B. bambos*, with its flowering means in several areas like Dansai, Phurua and Muang districts and giant thorny bamboo, with its dense culms mean there is little interest in these species and so they are seldom grown. The Hok variety, *D. hamiltonii*, a giant woody bamboo that originates around the junctions of streams, is less frequently cultivated due to its larger size, thick culms and difficulty in propagation using its rhizomes. *D. hamiltonii* generally had only a few culms per colony with approximately five colonies of 14–20 culms in a surveyed area of 60 m² along the river stream basin in Ban Nam Phoo village. Bamboos have different growth rates and development patterns depending on the species. Most Loei bamboo is greater in size, with higher numbers of culms and tillers and greater above and underground biomass than those grown on flat and mountain terrain. *D. hamiltonii* and *B. vulgaris* grow where there is high moisture availability that promotes elongation of rhizomes and root production due to the adequate moisture patches, high soil fertility and optimal soil physical properties. Conversely, mountain species including Rougwán, *T. siamensis* and *B. oliveriana* have fewer underground organs where logging has occurred and a slower growth rate.

Mountainous regions

There were 25 species from 8 genera and 4 unidentified species in this region, as presented in Table 3. Most genera and species were naturally distributed in the foothills and mountainous areas. There were seven species of Bambusa (*B. bambos*, *B. tulda*, *B. vulgaris*, *B. vulgaris f. waminii*, *B. multiplex*, *B. burmanica* and *B. oliveriana*). In this region, there was greater richness of *D. membranaceus*, *D. sericeus*, *D. hamiltonii* (Hog), *D. hamiltoni* (Hogwan) and *D. asper* than in the flat region Two species each were identified of the generar *Vietnamosasa*, *Schizostachyum* and *Neohouzeaua* including *V. ciliata*, *V. pusilla*, *S. virgatum*, *S. pergracile*, *N. mekongensis* and an unidentified *Neohouzeaua* species (Sodlaidang). One species each of *Gigantochloa*, *Thyrsostachys*, *Melocalamus* were identified. *N. mekongensis* has erect bamboo culms, thin-walled internodes, lanceolate leaf blades, medium-sized foliage leaf blades, and small foliage leaf auricles with long bristles (Dransfield et al., 2003). *M. compactiflorus*, a caespitose bamboo, is an elegant climbing bamboo, with a small lumen, grayish-green, rough, scandent, spreading culms, prominent swollen and whitish large nodes with several branches in a horizontal line. It is persistent, hard, brittle, cylindrical, covered with white appressed hairs and has persistent culm sheaths (Seethalakshmi and Muktesh, 1998).

Notably, some mountainous bamboo species resembled those found in the flat and river basin areas but were smaller in size.

Among the unidentified species, there were four bamboo varieties (Rougwán, Kama, Serm and Por). Rougwán has the most obvious phenotypic similarity to *T. siamensis* both in the phylogenetic tree and among its phenotypic traits (leaves, culms, nodes, internodes and rhizomes), except that it has dark purple culms with light striped sheaths surrounded with dark brown hairs. Additionally, Rougwán has young shoots with a sweet taste that can be consumed fresh and is not bitter like Roug. Kama is a medium-sized bamboo that is 5–10 m in height, with a diameter of 5–10 cm with 20–40 cm internodal length. Its culm shape characteristics are similar to those of *D. membranaceus*, but the culm wall is thicker than that of Sang. The dense culm with limbs and leaf hairs are similar to those of *G. albociliata*. However, the phylogenetic tree shows Kama related to *D. membranaceus*, *G. albociliata* and *B. multiplex*. Serm has characteristics similar to those of *S. pergracile* and *S. virgatum*, including long erect culms with long internodes, thin culms and is 10–15 m in height. Additionally, it has fresh, crispy shoots produced from nodal buds and rhizomes that appear with a purple sheath covered by dark brown hair of fresh shoots and a clear sheath scar along the node and oral setae. Por has small culms with light walls and was growing in mountainous areas along the border of the Muang and Thali districts after a mass flowering in 2014 to 2015. Light-green Por seedlings were found with small erect and thin culms and no thorns. The culms were 2–4 cm in diameter with 10–20 cm internodal length and limbs at the bottom of the culms. Por has short-necked pachymorph rhizomes, young light-green shoots with lacuna that appear inside the culm and culm sheaths that drop off easily. During the current survey, two local bamboo varieties of *B. multiplex* were less widely distributed and faced possible extinction in the river basin area.

The genetics of native bamboo are influenced by the interaction of topography, climate soil and their phylogenetics (Ohrnberger, 1999). Most native bamboos can adapt to growth under changing climatic patterns in various habitats and topography. This makes the bamboo biodiversity of Loei one of richest in Northeastern Thailand (Yospanya, 2007). A survey was conducted in 2008 by the Thailand Forest Survey Team (2009). They found 31 bamboo species, with 26, 19, 12, 12 and 7 species in the northern, northeastern, central, eastern and southern regions of the county, respectively. However, based on interviews with people in Loei province regarding local bamboo resources, there was reportedly greater species richness and densities last century due to climate being

optimal for bamboo growth. However, the changes since then in agricultural policy and infrastructure have resulted in more than 15 species declining in distribution in the surveyed areas, with national forest affected by deforestation in Loei. Thus, there is a real danger of some bamboo species facing extinction and many are endangered. During 1995–2019, several bamboo

species were extensively destroyed due to these factors, such as Rougwan, *M. compactiflorus* (Kaiwan), *S. virgatum*, *S. pergracile*, *D. hamiltonii*, *B. oliveriana*, *B. multiplex*, *B. blumeana*, *G. ligulata*, Sangong, *V. pusilla* and *B. tulda* from Loei. All of these are now in endangered, while Kama, Serm and Por bamboo types are facing extinction.

Table 3 Surveyed bamboo species in mountainous regions of Loei province

No.	Local name	Species	Distribution
<i>Bambusa</i> genus			
1	Pa	<i>B. bambos</i>	Foothills in Phurua, and Dansai districts
2	Bongpa	<i>B. tulda</i>	Mixed deciduous forest and dry evergreen forest
3	Luang, Sangkum	<i>B. vulgaris</i>	Muang district
4	Namtao	<i>B. vulgaris</i> f. <i>waminii</i>	Village and government offices
5	Sangprailek	<i>B. multiplex</i>	Hilly areas and foothills
6	Bongwanmuangloei	<i>B. burmanica</i>	Dansai district
7	Ruaghangchang	<i>B. oliveriana</i>	Boundary of Muang, Tali, Phurua and Dansai districts
<i>Dendrocalamus</i> genus			
8	Sang, Sangnual	<i>D. membranaceus</i>	Hilly areas in the Muang district
9	Sangmon	<i>D. sericeus</i>	Mountains, houses, and resorts
10	Hog	<i>D. hamiltonii</i>	Foothills, mountains of Phurua and Dansai districts
11	Hogwan	<i>D. hamiltonii</i>	Foothills in Phurua and Dansai districts
12	Tongpa	<i>D. asper</i>	Little found upland and in foothills
<i>Gigantochloa</i> genus			
13	Rai	<i>G. albociliata</i>	Foothills, waterside, ridge, mix with other species and plants
<i>Thyrsostachys</i> genus			
14	Roug	<i>T. siamensis</i>	Foothills, waterside, ridge, farm (grown for consuming shoots and other uses)
<i>Vietnamosasa</i> genus			
15	Jode	<i>V. ciliata</i>	Foothills, ridge and mountains in Muang, Phurua and Dansai districts
16	Pek	<i>V. pusilla</i>	Foothills, ridges, and mountains in Muang and Dansai districts
<i>Schizostachyum</i> genus			
17	Hia	<i>S. virgatum</i>	Foothills, waterside, ridges of dry and hilly evergreen forest
18	Kaolam	<i>S. pergracile</i>	Hills, tributary edges, Loei riverside, big culms found
<i>Melocalamus</i> genus			
19	Kaiwan, Wan, Saiwan, Saiton	<i>M. compactiflorus</i>	Phurua district and Koksaton, Dansai district
<i>Neohouzeaua</i> genus			
20	Sod, Lod	<i>N. mekongensis</i>	Among hills and mountains in Muang, Phurua and Dansai districts
21	Sodlaidang	<i>N. sp.</i>	among hills, and Koksaton and Dansai districts
Unidentified genus			
22	Kama	-	Among hills, little found in Muang district and Koksaton and Dansai districts, 1–2 colonies per point
23	Serm	-	Among hills, little found in some areas in Muang and Dansai districts, 1–2 colonies per point, common in hills
24	Por	-	Rainforest at boundary of Muang and Tali districts
25	Rougwan	-	Muang district introduced from Phukradung district

Genetic diversity

Polymorphic bands have different genetic distances that can be utilized to provide discriminating power for studying plant diversity. Genetic diversity simply reflects differences in morphological data. AFLP has been applied for taxonomic studies of bamboo, including in infrageneric studies in *Phyllostachys* (Hodkinson et al., 2000), and in suprageneric studies in old world tropical bamboos (Loh et al., 2000). In the current study, 10 randomized AFLP primer combinations were used among extracted DNA samples of the 22 species. Polymorphic markers were used to analyze the genetic diversity of the bamboo and 179 monomorphic bands were identified with a mean of 17.9 bands per marker and 8.14 bands per species (data not shown). Similarly, research on genetic variation and relationships among a subtribe of Bambusinae using eight AFLP primers with 15 bamboo species found 128 monomorphic bands with a mean of 16 bands per marker and 8.53 bands per species (Loh et al., 2000).

The AFLP bands shown in Fig. 3 were used for analysis of the relationships among bamboo species using the NTSYSpc 2.02J package. Then, a dendrogram was constructed and is presented in Fig. 4. In this dendrogram, a Jaccard's similarity coefficient of 0.265 was used as a standard value of

classification. The dendrogram could be split into three groups. There were eight species from four genera in Group 1 consisting of *Bambusa*, *Dendrocalamus*, *Gigantochloa*, *Thyrsostachys* and an unidentified genus. In Group 2, there were seven species of four genera and one unidentified genus in Group 2, comprising *Bambusa*, *Gigantochloa*, *Neohouzeaua* and an unidentified genus. Group 3 had three species of *Dendrocalamus*. However, the current clustering was different in terms of the number of groups compared to other research focusing on phylogenetic relationships among 15 bamboo species, namely 7 species of *Bambusa*, 5 of *Gigantochloa*, 2 of *Dendrocalamus* and 1 of *Thyrsostachys*, using AFLP that identified only two clusters (Loh et al., 2000). They grouped four species of *Bambusa*, five of *Gigantochloa* and one of *Dendrocalamus* in Group 1, three of *Bambusa* in Group 2 and the other species were ungrouped. The two different clusters for *Bambusa* species suggested that this genus is polyphyletic and highlights the potential of AFLP techniques in assessing the variation and relationships within *Bambusa* species (Loh et al., 2000).

In the current study, six species of the *Bambusa* genus were in two groups. Group 1 contained two varieties of the species, *B. nana* and *B. oliveriana*, while Group 2 accounted for four species (*B. longispatha*, *B. burmanica*, *B. bambos* and *B. vulgaris*). Similar research on 15 bamboo species

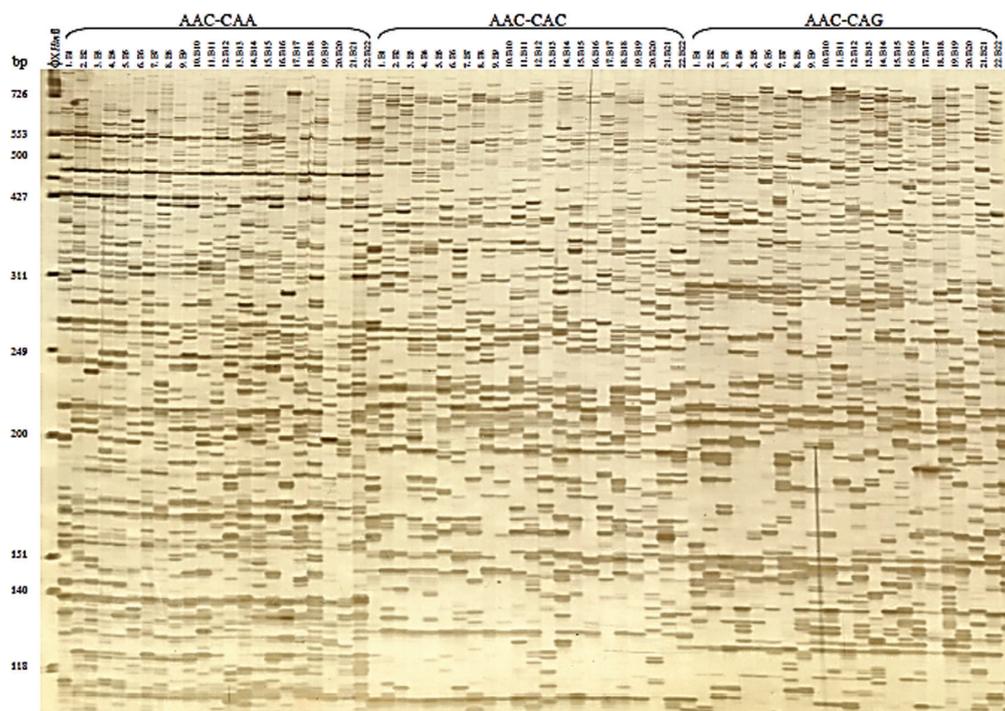


Fig. 3 Amplified fragment length polymorphism profiles of primers, AAC-CAA, AAC-CAC and AAC- CAG, visualized using polyacrylamide gel electrophoresis with silver staining

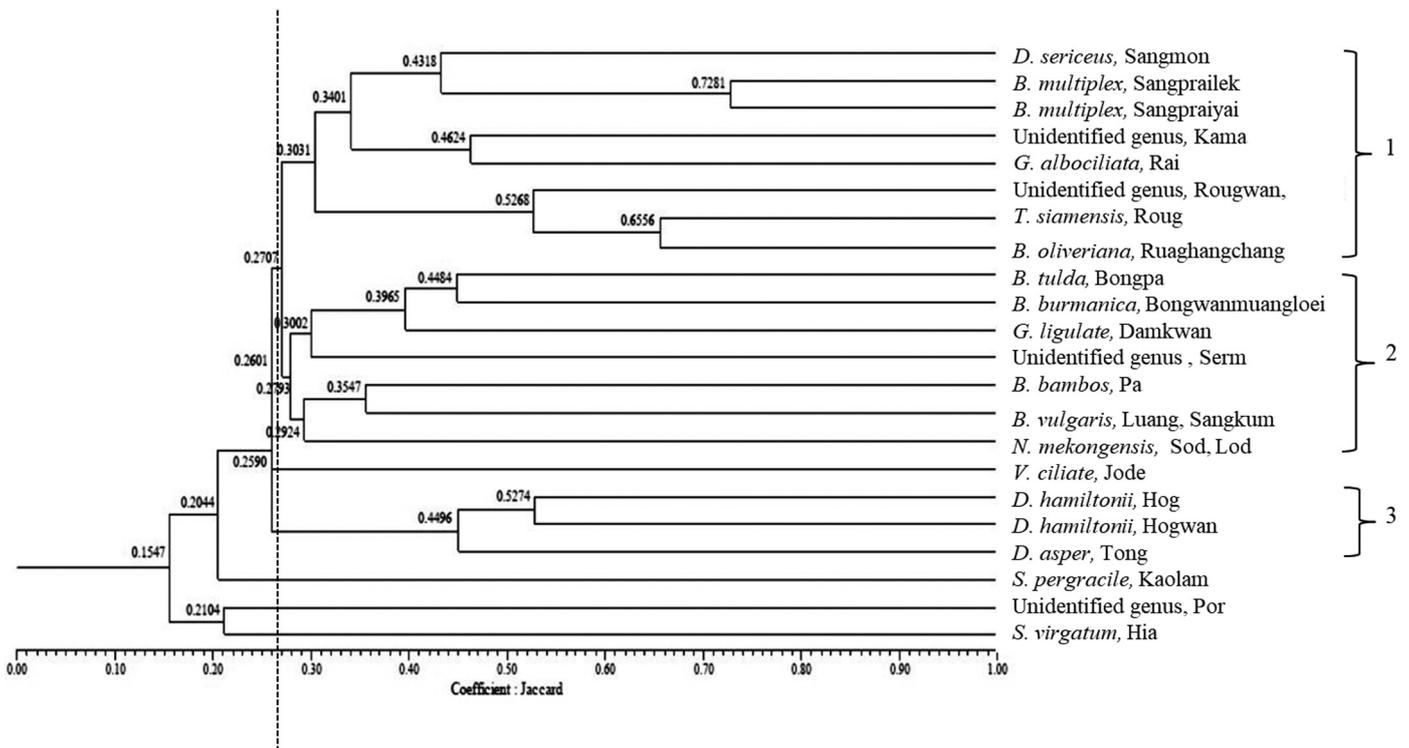


Fig. 4 Phylogenetic tree of 22 bamboo species with 10 amplified fragment length polymorphism markers based on Euclidean distance dissimilarity method and grouping using unweighted pair group method with arithmetic mean in NTSYSpc 2.02J, where the tree was split into three groups (1, 2 and 3) composed of 8, 7 and 3 species, respectively, and 4 species without a group

indicated that the *Bambusa* genus had four genera with eight AFLP primers in two groups (Loh et al., 2000). Two species of the genus *Gigantochloa* (*G. albociliata* and *G. ligulata*) are genetically related to the *Bambusa* genus, as they are in both Groups 1 and 2. The results were quite similar to Loh et al. (2000). Vorontsova et al. (2016) and Vorontsova (2018) found that five species of *Gigantochloa* formed a single cluster that diverged from a cluster of *B. tulda*, *B. ventricosa* and *B. vulgaris*. However, three species of *Dendrocalamus* (*D. hamiltonii* (Nee & Arn.Ex Munro), *D. hamiltonii* and *D. asper*) were genetically related in Group 3. Only one species in Group 1 (*D. membranaceus*) was examined. Therefore, the *Bambusa* and *Gigantochloa* species were in two groups because both genera were included under the subtribe Eubambuseae, while *Dendrocalamus* species were included within the subtribe, Dendrocalameae (Das et al., 2007). *D. sinicus* has diversity in its two types of culm shapes (bent and straight culms) (Dong et al., 2012). According to Ramanayake et al. (2007), the genetic distances separating *D. longispatus* from *D. giganteus* and *D. asper* were 0.577 and 0.672, respectively. Additionally, Das et al. (2017) performed clustering analysis on 125 accession numbers of an indigenous species, *D. strictus*

that could be divided into three groups. The two different clusters for *Bambusa*, *Gigantochloa* and *Dendrocalamus* in the current research suggested that these genera are polyphyletic and highlights the potential of AFLP techniques in assessing the variation and relationships within genera (Loh et al., 2000). Thus, *D. membranaceus* was not clustered with the *Dendrocalamus* species in Group 3 but was clustered with Group 1 instead. In the current classification, two species (*S. pergracile* and *S. virgatum*) in the genus were genetically different. Additionally, *V. ciliate* and an unidentified species known by the local name, Por, were not related to any group. The Jaccard similarity of the 10 AFLP markers among 22 species was in the range 0.1273–0.7281, whereas the *B. nana* Robx and *B. nana* hort, were the most highly related with a Jaccard similarity of 0.7281. The current study found a low Jaccard similarity of 0.1273 for *B. nana* and *S. virgatum*. The cophenetic correlation (r) of the 22 species with the 10 AFLP markers was 0.9279. A cophenetic correlation of more than 0.9 indicates a high degree of grouping.

The outcome of this research provided the identification of species and genera for bamboo certification and a core collection of plant genetic material for a conservation project

under the Royal Initiative of Princess Maha Chakri Sirindhorn. Additionally, data from the survey consisting of distribution, as well as the numbers of local genera and species of bamboo in Loei province, will be important data for future conservation planning. The implications of the study and recommendations for further research will focus on evaluation of DNA fingerprinting of local bamboo species, germplasm collection of the species and development of cooperation between local government officers, the Royal Forestry Department and various research universities for planning and executing bamboo conservation for sustainable utilization.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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